

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

Report of TCDD Analysis in Spring River Fish

Daniel J. Harris  
Sanitary Engineer, EP&R/ENSV

Dick Smith, WMBR/ARWII

THRU: William J. Keffer  
Chief, EP&R/ENSV

John C. Wicklund  
Director, ENSV



40032113  
SUPERFUND RECORDS

JDB

Site:	Spring River
ID #	11602607450131
Break:	3.3
Other:	8/8
	4-82

0751

This memorandum, which reports the TCDD data resulting from the second round of sampling and analysis of fish tissue in the Spring River, should be considered as a supplement to "Report-Preliminary Investigation of Spring River Basin" date of March 22, 1982. These data were provided by Dr. Mike Gross, University of Nebraska, on fish collected December 28, 1981. Stream mileage (measured upstream from confluence with Lake of the Cherokees), sampling point description, type of fish analytical results and detection limits are as follows:

Stream Mileage	Description	TCDD PPT	Detection Limit PPT
22.04	Highway 166 at Baxter Springs, Kansas. Sample No. SD5002-1-5. A composite sample consisting of five Carp for whole fish analysis.	ND*	2.6
49	County road bridge crossing near Gaylesburg, Missouri. Sample, No. SD5010-13-15. A composit sample consisting of three Northern Hog Suckers for whole fish analysis.	ND	1.3
72.1	Head dam at Morrow Milling near Carthage. Sample No. SD5008-24-30. A composite sample consisting of six Spotted Suckers for whole fish analysis.	ND	0.79
82.1	County road bridge at LaRussell. Sample No. SD5004-18,20-24. A composit sample consisting of four White Suckers for whole fish analysis.	ND	1.2
	Location same as above. Sample No. SD5004-19,21,23,25. A sample consisting of four White Suckers for fillet analysis.	ND	0.92

*Sept 17/82*

Not detected DSH: 4-7-82

SYMBOL	EP&R	EP&R	ENSV	CONCURRENCES			
SURNAME	W. J. Keffer	D. J. Harris	D. Smith				
DATE	4-07-82	4-07-82	4-8-82				

0188A

118            Celia's Spring River Trout Farm upstream            ND\*            0.81  
Verona. Sample No. SD5003-1-5. A sample  
consisting of five Rainbow Trout for whole  
fish analysis.

Analytical results on TCDF\*\* isomers, which Professor Gross has indicated as being present, are expected in a few days. A copy of the analytical report is attached.

Further distribution of these data are the responsibility of ARMM. It would seem appropriate to delay public release of these data until such time as the fish data from Dr. Stallings, via Missouri Department of Conservation, is available.

Attachment

cc: Dan Harris, EP&R/ENOV  
John Zirschky, E&E

\*Not detected

\*\*Tetrachlorodibenzofurans

## UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

DATE: April 6, 1982

SUBJECT: Report of TCDD Analysis in Spring River Fish

FROM: Daniel J. Harris  
Sanitary Engineer, EP&R/ENSV

TO: Dick Smith, WMBR/ARWM

THRU: William J. Keffer  
Chief, EP&R/ENSVJohn C. Wicklund  
Director, ENSV

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cc: Dan Harris, EP&R/ENSV  
John Zirschky, E&E

\*Not detected

\*\*Tetrachlorodibenzofurans

# UNL

The University of Nebraska-Lincoln

Department of Chemistry  
Lincoln, NE 68588

March 19, 1982

Dr. Deborah Leoris Rosengren  
Sample Management Office  
VIAR and Company, Inc.  
P. O. Box 818  
Alexandria, VA 22313

*Rec'd.  
3/22/82  
Environmental  
Spring  
SD 50*

(Ref: Project 98G)

Dear Deborah:

Enclosed please find a report on the analysis of fish for TCDD and TCDF.

The analysis for the total concentration of TCDD were carried out by capillary column GC/HRMS. No TCDD was detected in samples by either method. However, capillary column GC/MS data will be presented since we obtained better detection limits by this method.

We also wish to inform you that the samples do contain various TCDF isomers. The results of those analyses will be reported to you within a few days.

If you have questions on this report, please call either Dr. N.C. A. Weerasinghe or me (402/472-3507).

Sincerely yours,



Michael L. Gross  
Professor

MLG:lc  
Enclosure

Report

**Analysis of Tetrachlorodibenzodioxins  
and Tetrachlorodibenzofurans in Fish**

**Project 98G**

to

**VIAR & Company  
114 North Columbus Street  
Alexandria, VA 22314**

by

**Midwest Center for Mass Spectrometry  
Department of Chemistry  
University of Nebraska-Lincoln  
Lincoln, NE 68588**

**Michael L. Gross  
Professor & Director**

**Date**

Sample Extraction Procedure for Tissue

A 1-10g sample was accurately weighed and spiked with a known amount (2.0-2.5ng) of  $^{13}\text{C}_{12}$ -TCDD. It was then saponified in 15ml of ethanol\* and 30ml of 40% aqueous KOH in a reflux apparatus for 60 minutes with stirring. The sample should be completely hydrolyzed before terminating the saponification.

The solution was transferred to a 250ml separatory funnel and diluted with 20ml of ethanol and 40ml of water and extracted four times with nanograde hexane. The first extraction was done with 25ml of hexane, shaking vigorously for one minute. The lower aqueous layer was removed to a clean beaker, and the upper hexane layer was decanted to a 125ml separatory funnel. The aqueous layer was then extracted three times more with 15ml portions of hexane, each time adding the hexane to the 125ml separatory funnel. The combined hexane extracts were washed with 10ml of water to remove excess base.

The combined hexane extracts were washed 4 times with 10ml concentrated  $\text{H}_2\text{SO}_4$ , or until both layers were clear. As many as 8 extracts may be necessary, depending on the sample. Again the hexane was washed with 10ml of water. The hexane layer was decanted to a 2 ounce jar and concentrated under a stream of dry nitrogen to approximately one ml.

Two chromatography steps were done, the first being a silica gel column. No activation of silica was necessary. A 5cm column was prepared using a disposable pipet plugged with glass wool. The silica was capped with 1/4cm anhydrous sodium sulfate to remove water, and then wetted with hexane. The sample, dissolved in one ml of hexane,

was transferred to the column. A second ml of hexane was used to rinse the jar and was subsequently added to the column. Dioxin was eluted with three ml of 20% (V/V) benzene in hexane. All eluate was collected in another 2 ounce jar and concentrated to a volume of one ml.

Alumina was washed by saturating with methylene chloride, removing excess solvent, then activating at 225°C for 24 hours. A column was prepared in the same manner as the silica column above. The column was cooled to room temperature in a desiccator before use.

Hexane was used to wet the column before transferring the sample. The jar was again rinsed with one ml of hexane which was transferred to the column. The alumina was eluted with two 3 ml portions of pesticide grade  $\text{CCl}_4$ , then with 4ml of  $\text{CH}_2\text{Cl}_2$ . These solvents were used to rinse the jar before being transferred to the column. The dichloromethane fraction, which contained the TCDD, was collected in a centrifuge tube, and the solvent was evaporated to a small volume under a stream of dry nitrogen. The sides of the centrifuge tube were rinsed down with one ml hexane and again the volume was reduced. The tube was rinsed a final time with one ml of hexane and the solvent evaporated until the volume was less than 100ml. The centrifuge tube was capped with a teflon-lined screw cap and stored in a freezer at about -20°C until analysis.

List of Materials Used in Tissue Extractions

Acetone, OmniSolv<sup>\*</sup>, MCB

Benzene, OmniSolv, MCB

Carbon tetrachloride, OmniSolv, MCB

Ethyl alcohol, OmniSolv, MCB

Hexane, OmniSolv, MCB non UV

Methylene chloride, OmniSolv, MCB

Sulfuric acid, concentrated, analytical reagent, Mallinckrodt

Water, distilled in glass

Potassium hydroxide, analytical grade, Mallinckrodt

Sodium sulfate (anhydrous), analytical grade, Fisher

Aluminum oxide, neutral, activity grade I, Woelm Pharma

Silica gel, 60-200 mesh, reagent grade, Baker Chemical Co.

Dry nitrogen (boil-off from liquid N<sub>2</sub>)

<sup>\*</sup>All OmniSolv line solvents are distilled in glass, suitable for chromatography and residue analysis.

## TCDD and TCDF Analysis by Capillary Column GC/HRMS

Appropriate dilutions of the samples were made with hexane at the time of analysis and the aliquots from the resulting solutions were used for capillary column GC/HRMS.

### A. Gas Chromatography/Mass Spectrometer

A Kratos MS-80 medium resolution mass spectrometer (ultimate resolution 20,000), equipped with a 5 channel multiple peak monitoring (MPM) device was used. The mass spectrometer was coupled to a Carlo-Erba Gas chromatograph. The gas chromatograph was equipped with an SE-54 fused silica capillary column (0.25 mm x 30 m) which was coupled directly with the ion source.

### B. Gas Chromatographic Conditions

Typical operating conditions were: Helium with a linear velocity of 35 cm/sec, injector 250°C, detector 275°C. Two temperature programs were utilized in the isomer specific analysis of TCDD and TCDF. For TCDD the GC parameters were: column temperature 200-280°C, programmed at 5°/min to 280°C. For TCDF analysis the conditions were: column temperature 150-280°C, programmed at 5°/min to 280°C. A splitless injection technique was utilized for both types of analysis. The sample was injected in hexane at a temperature of 57°C. The split/sweep valves were kept closed for 2 minutes after injection.

### C. Mass Spectrometric Conditions and Multiple Ion Selection

The mass spectrometer was operated in the EI mode (70eV, 250°C) at 7500 resolving power. (For TCDD a resolving power of 3000 was used). Peak profiles were acquired at an amplifier bandwidth of 30,000Hz. For the TCDD analysis, the ions  $m/z$  319.8965,  $m/z$  321.8936 and  $m/z$  333.9339 ( $^{13}\text{C}$ -2,3,7,8-TCDD) were monitored on three channels using the MPM. The instrument was tuned using  $m/z$  330.9792 of PFK, and this ion was used as a check mass on channel 4. For the TCDF analysis,  $m/z$  303.9016,  $m/z$  305.8986 and  $m/z$  333.9339 were monitored on 3 channels of the MPM. The instrument was tuned using the PFK  $m/z$  304.9824 which was used as a check mass. The output of the mass spectrometer was recorded on a 3-pen strip chart recorder (Linear Model-595).

### D. Calculation of Results

Quantification was achieved using the internal standard ratio method. Throughout the experiment, standard samples containing 2,3,7,8-TCDD or 2,3,7,8-TCDF and  $^{13}\text{C}$ -2,3,7,8-TCDD were analyzed. The slopes of the calibration plots were taken as the averages of the ratios of  $(I^{334}/\text{ng})/(I^{322}/\text{ng})$  ( $I$  is the normalized intensity for the designated mass) for TCDD and  $(I^{334}/\text{ng})/(I^{306}/\text{ng})$  for TCDF obtained using the standard samples.

Residues of TCDD or TCDF in actual samples were calculated by comparing the ratios of intensities of  $I^{332}/I^{334}$  (for TCDD) and  $I^{306}/I^{334}$  (for TCDF) obtained for a given sample with the slope of the calibration plot. The detection limit was considered to be the respective value obtained for an intensity of 2.5 x noise level

measured at the base line. The peak height of  $m/z$  322 was used in estimating the percentage (fraction) of any given isomer present in TCDD analysis. Similarly,  $m/z$  306 was used for this purpose in the TCDF analysis. The percentage of any given isomer represents the fraction of the isomer in question (as represented by the peak height of  $m/z$  322 or  $m/z$  306 for TCDD and TCDF respectively) compared with the sum total of all the peak heights detected.

The retention times of the isomers were measured from the point of injection and normalized to the position of the signal of the internal standard,  $^{13}\text{C}$ -2,3,7,8-TCDD.

The internal standard ( $^{13}\text{C}$ -2,3,7,8-TCDD) was utilized in the calculation of percent recoveries, and in doing so the absolute intensity ( $I^{334}$  normalized) was measured and compared with the intensities ( $I^{334}/\text{ng}$ ) obtained by injecting standard solutions of the internal standard. The recovery for TCDF was assumed to be identical to that for TCDD according to this calculation method.

Table: Capillary Column GC/HRMS Analysis of Tissues from Fish for TCDD

Sample Code	Weight (g)	Weight of Spike (ng)	Concentration (ppt)	Detection Limit (ppt)	Percent Recovery
SD5002	10.74	2.55	nd	2.60	20
SD5003	10.69	2.50	nd	0.81	55
SD5004 (w)	11.11	2.45	nd	1.20	30
SD5004 (f)	10.09	2.50	nd	0.92	45
SD5008	12.44	2.55	nd	0.79	40
SD5010	11.43	2.50	nd	1.30	35
SD-Fish Blank	12.55	2.55	nd	0.44	65

ppt = parts-per-trillion